

Exhibit C

NOTEBOOK NO. 2369
ISSUED TO Randy Saki
ON 11-11-1988 **19**
DEPARTMENT Human Genetics
RETURNED 19

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STEVENVILLE, MICHIGAN 49127

From Page No. X

Rec'd 80 μ l Tag polymerase from David Gelfand.
 Tube is labeled "fraction VIIA", will call it lot 3B.
 (Shirley and David have 3A, more concentrated.) This
 stuff is at 10 μ g/ μ l using their activated salmon
 sperm (or is it calf thymus) DNA assay. Titrate
 for PCR amplification.

A, F: 1 μ l per 100 μ l rxn.

B, G: 1/2 " "

C, H: 1/4 " "

D, I: 1/8 " "

E, J: 1/16 " "

A-E: Molt4

F-J: GM2064

Molt4 and GM2064 @ 100 μ g/mlPC03 and PC04 @ 10 μ M, dNTP @ 10mM each35 μ l Molt4 or GM206435 μ l 10x Tag salts35 μ l PC0335 μ l PC0435 μ l DMSO52.5 μ l dNTP122.5 μ l H₂O350 μ l → 5', 95°

Cool to RT and divide into one 100 μ l ~~volume~~ and
 four 50 μ l ~~samples~~ samples (50 μ l left over). Add 1 μ l Tag
 polymerase (lot 3B, 10 μ g/ μ l) to the 100 μ l volume and
 mix. Prepare four 50 μ l V serial dilutions in four
 remaining samples. ^{sample} two-fold

Final concentrations of enzyme per 100 μ l reaction
 volume: 1 μ l, 1/2, 1/4, 1/8, 1/16.

Overlay with mineral oil

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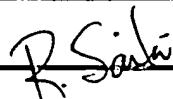
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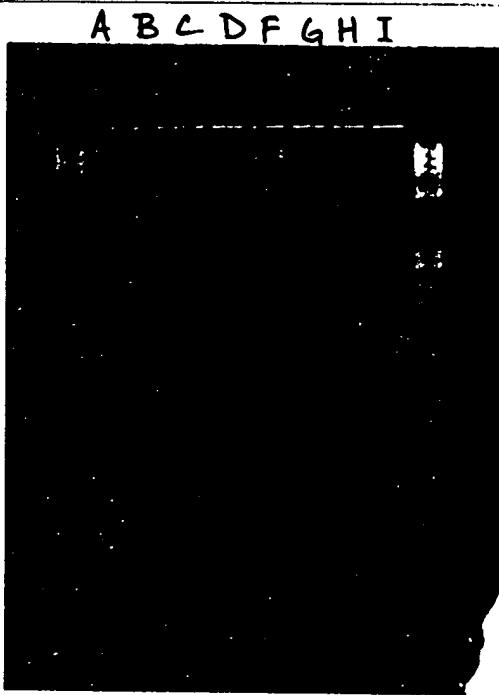
Save remaining 50 μ l (1/16 dil'n) and store @ 4° (just in case enzyme works at < 1/16 μ l per 100 μ l).

Subject to 24 cycles: 2 min ramp, 35° to 95°
2 min ramp, 95° to 35°

After last cycle, incubate additional 5 min at 65° to complete final (25th) extension.

Extract oil with CHCl_3 .

Load 5 μ l each A-D and F-I on 4% NuSieve/0.5% agarose (1xTBE).



*Molt4, Molt4, Molt4, Molt4
GM2064, GM2064, GM2064*

Got PCR product in Molt4 at all four dilutions, even 1/8 μ l! As expected, nothing in GM2064.

Background is very low, virtually nonexistent for 1/4 and 1/8 μ l samples. Maybe combination of enzyme and "fast ramp" protocol is responsible.

Need to check 1/16 μ l samples; might be band there, too.

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Compare titrations of lot 2A and lot 3B of Tag polymerase using "Pro/Pette" protocol.

A I: 1 μ l
 B J: $\frac{1}{2}$
 C K: $\frac{1}{4}$
 D L: $\frac{1}{8}$

E M: $\frac{1}{16}$ μ l
 F N: $\frac{1}{32}$
 G O: $\frac{1}{64}$
 H P: $\frac{1}{128}$

A-H: lot 2A
 I-P: lot 3B

50 μ l Melt4
 50 μ l 10X salts
 50 μ l PCO3
 50 μ l PCO4
 50 μ l DNISO
 75 μ l dNTP
175 μ l H₂O
500 μ l \rightarrow 10', 95°

Prepare eight 50 μ l two-fold serial dilutions with 1 μ l lot 2A or lot 3B as described on page 88.

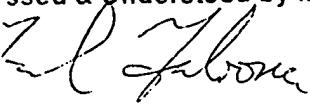
Subject to 24 cycles in ProPette: 2 1/2' min ramp, 37 to 95°
 3' min ramp, 95 to 37°

After last cycle incubate 5' @ 60° to complete 25th cycle extension.

Load 5 μ l each onto 4% NuSieve/0.5% agarose/1x TBE.

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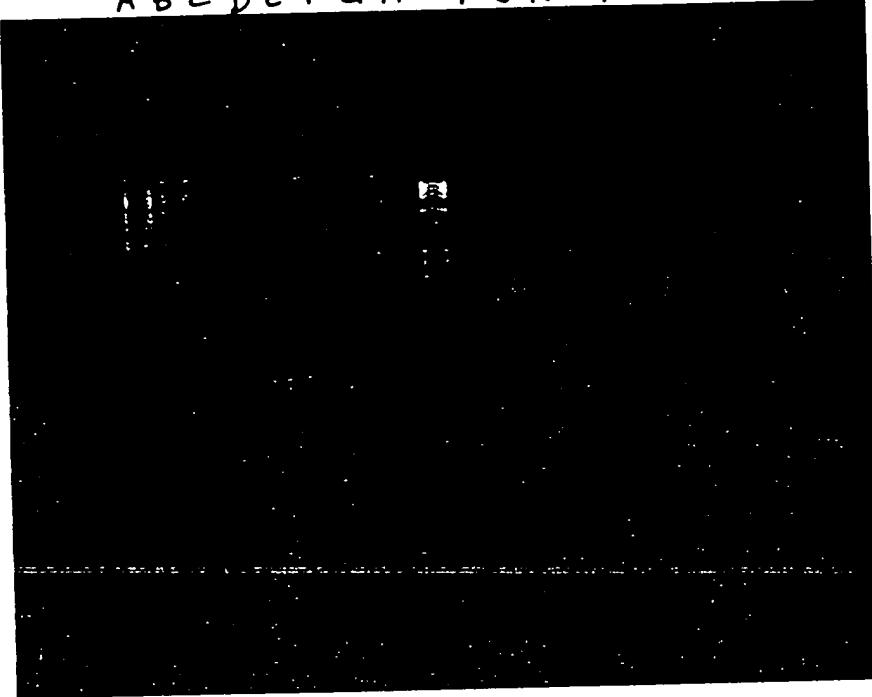


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A B C D E F G H I J K L M N O P



amplification

Don't see a cut-off in lot 2A anymore. Can see a band is far down as $1/64 \mu\text{l}$ (lane G). Also, best S/N ratio ratio is at $1/16 \mu\text{l}$ instead of $1/8 \mu\text{l}$.

Lot 3B seems to peak at $1/2 \mu\text{l}$ little although this should be rechecked. (Especially ~~recheck~~ ^{since} photo on page 1649 shows peak at $1/16 \mu\text{l}$ and $1/8 \mu\text{l}$)

There seems to be more background in these samples than in those done "fast ramp". ~~Compare~~ Need to compare on same gel to be sure.

This gel show effect of polymerase on S/N ratio quite nicely.

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Lot 3 Tag polymerase seems to be losing activity. Initially, optimal concentration (for PC03/04) was at 2.5 μ l per 100 μ l reaction. Over the last 2-3 weeks activity has gradually ~~blown up~~ dissipated. Most recent attempts have failed (not recorded). Only a very weak PCR products product was seen with 5 μ l. Russ, Dory, and Steve have had similar experiences.

Unlike lot 2A, the storage buffer for lot 3 does not contain the non-ionic detergents Tween 20 or NP 40.* David Gelfand's experience with this polymerase indicates that it is a sticky enzyme and he routinely uses both detergents during purification to improve yield and during assay to stimulate activity.

May be that in the absence of detergents and at 20° the enzyme is aggregating. Addition of "soap" to either the storage buffer or the PCR reaction may restore activity. Will try the latter first.

	μ l/100 μ l	
A H :	1/2	
B I :	1/4	A-G: (-) detergent
C J :	1/8	H-N: (+) detergent (0.05% each)
D K :	1/16	
E L :	1/32	
F M :	1/64	
G N :	1/128	

* Another difference is that lot 3 contains 200 μ g/ml gelatin. Lot 2 doesn't.

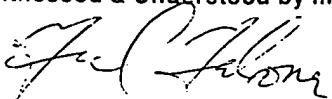
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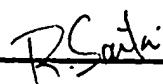
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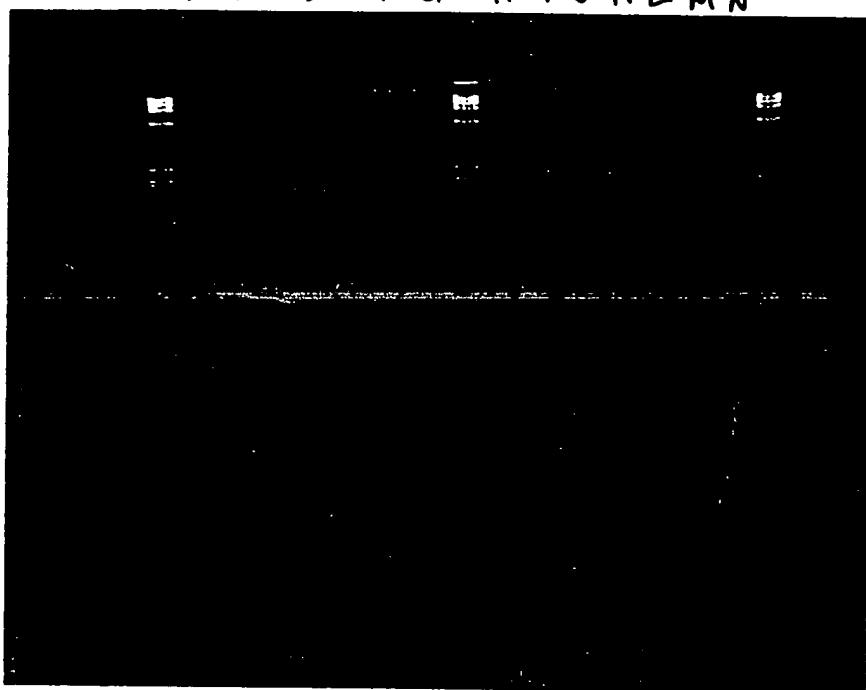


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Page No. 102

Load 5 μ l each onto 4% NuSieve / 0.5% agarose / 1x TBE
→ 100V, 90'



Detergents definitely have some effect. Without them can only see a band in 0.125% S_n sample (A). But with them, can see bands in S_n (H), 2.5 (I), and very faintly in 1.25 (K).

Although Tween and NP40 helped, activity still is not as good as it was originally (p. 81).

Maybe that adding more detergents or adding instead to the storage buffer will help be better.

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Page No. 101

Molt4 c. 100 μ g/ml, PC03 and PC04 @ 10 μ M
dNTP @ 40 mM (p99), Tween/NP40 @ 0.5% each

A-G: 50 μ l Molt4
50 μ l 10X salts
50 μ l PC03
50 μ l PC04
50 μ l DMSO
75 μ l dNTP
175 μ l H₂O
500 μ l \rightarrow 10' 95°

H-N: 50 μ l Molt4
50 μ l 10X
50 μ l PC03
50 μ l PC04
50 μ l DMSO
75 μ l dNTP
50 μ l Tween/NP40
125 μ l H₂O
500 μ l \rightarrow 10' 95°

Dispense each 500 μ l mix into one 100 μ l sample and six 50 μ l samples. Add 1/2 μ l lot 3 to the 100 μ l sample and dilute serially dilute, preparing seven 50 μ l two-fold serial dilutions: 1/2 μ l to 1/128 μ l.

Overlay with mineral oil and subject to 24 cycles on Sainsky's ProPette: 3' ramp 37° to 95° (hot water set at 102°)
3' ramp 95° to 37°

After last cycle, incubate additional 10' at 56°

Extract oil with CHCl₃. (Samples H-N became cloudy. Probably interaction of detergents with chloroform.)

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Follow up on expt. described page 101 by determining if adding detergents directly to enzyme stock is a better way to go. Gel fund suggests 0.5% each is a good starting point.

19 μ l Tag polymerase (lot 3, 10 4 μ l)

1 μ l 10% Tween 20 / 10% NP-40

20 μ l Tag pol, 9.5 4 μ l, 0.5% each detergent \rightarrow incubate @ RT

for ~10', mixing
thoroughly. Store
@ 4°

A E: 1
B F: $\frac{1}{2}$ } μ l Tag
C G: $\frac{1}{4}$ }
D H: $\frac{1}{8}$

A-D: Tag w/o detergent
E-H: Tag w/ detergent

reagents as desc. page 101

30 μ l Molt 4
30 μ l 10X salts
30 μ l PC03
30 μ l PC04
30 μ l DMSO
45 μ l dNTP
105 μ l H₂O
300 μ l \rightarrow 10', 95°

Prepare two 300 μ l mixes. Divide each into one 100 μ l sample and three 50 μ l samples. Add 1.0 μ l enzyme, with or without detergent, to the 100 μ l sample and serially dilute 50 μ l into the three 50 μ l samples.

Amplify and workup as described p 102 except use our Pro/Pette with this program: 2 1/2 ramp, $\frac{35}{3}$ ° to 95°
3' ramp, 95° to $\frac{35}{3}$ °

Block 15ml

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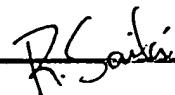
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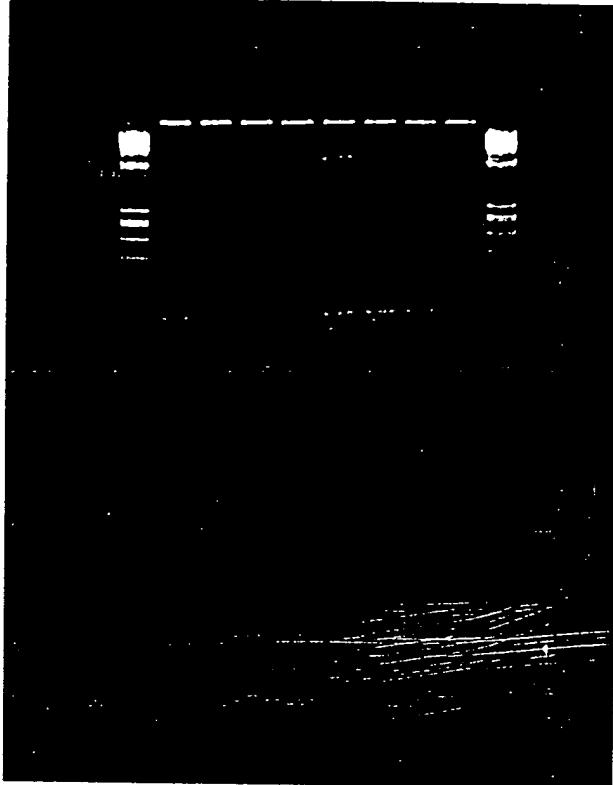


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On Page No. 104

Load 5 μ l each sample onto 4% NuSieve/0.5% agarose/1xTBE.
 → 100v

A B C D E F G H



This is it! Activity of enzyme with detergent is as good as (maybe even better) than original titration (see p. 81).

Based on this expt. best conc. of enzyme is either 5.0 μ l (F) or 2.5 μ l (G). Former may have a teeny bit more PCR product, but latter has less background. (Either is fuckin' good.)

Looks as if activity in (-) detergent enzyme has gotten even worse. Can barely see the 1 μ l sample (B).

Should add detergent to the remaining enzyme stock.

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